Supplementary material

Interplay between Reactive Oxygen Species and the Inflammasome are crucial for restriction of *Neospora caninum* replication

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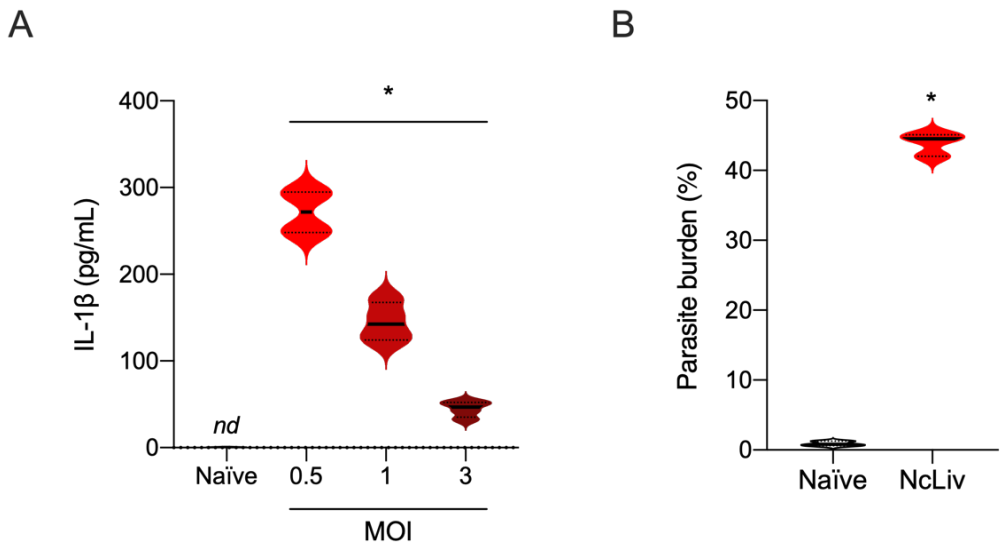
Legends:

Supplementary Figure 1. The multiplicity infection (MOI; cell:parasite ratio) 0.5 of *N. caninum* represented a good ratio to assess inflammasome activation in marrow-derived macrophages (BMDMs). BMDMs were infected for 18 hours with *N. caninum* tachyzoites *(*NcLiv) in different MOI (0.5, 1 and 3) and IL-1β production was measured by ELISA (A). BMDMs were infected for 18 hours with *N. caninum* tachyzoites *(*NcLiv) in MOI 0.5 and parasite burden was determined by flow cytometry using DDAO-SE (fluorescent ester-based probe) stained tachyzoites, represented in % of DDAO-positive cells (B). Values are representative of two independent experiments and each condition was conducted at least in triplicates. Values indicating mean ± SEM of cytokine levels in relation the standard curve and fluorescence percentage (*P < 0.05; ANOVA with the Bonferroni multiple comparison post-hoc test or t test between naïve and *N. caninum* infected BMDMs).

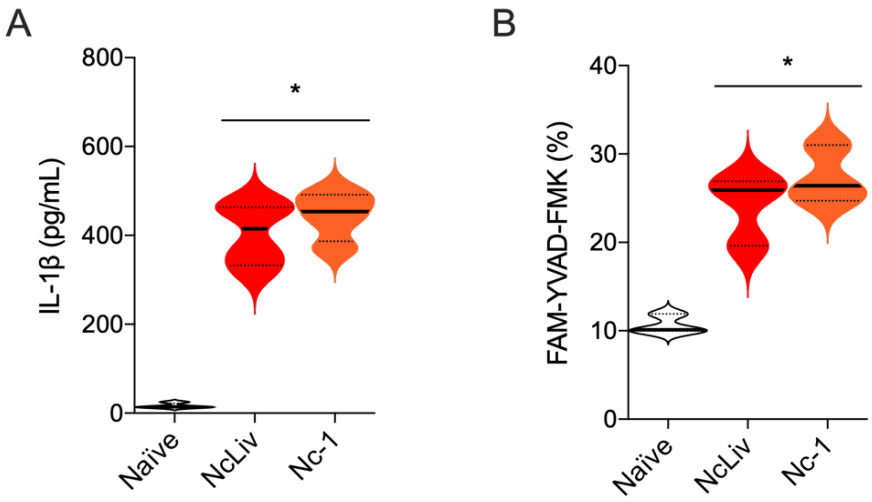
Supplementary Figure 2. Different *Neospora caninum* isolates induced similar phenotypes in the inflammasome activation. BMDMs were infected for 18 hours with *N. caninum* tachyzoites (NcLiv or Nc-1; MOI 0.5). IL-1β production was measured by ELISA (A) and Caspase-1/11 activity was assessed by flow cytometry using fluorescent FAM-YVAD-FMK (FLICA) probe, represented in % of FLICA-positive cells (B). Values are representative of two independent experiments and each condition was conducted at least in triplicates. Values indicating mean ± SEM of cytokine levels in relation the standard curve and CASP-1/11 activity (*P < 0.05; ANOVA with the Bonferroni multiple comparison post-hoc test between naïve and *N. caninum* infected BMDMs).

Supplementary Figure 3. Comparison of the inflammasome activation by *N. caninum* and known agonists of the pathway. BMDMs were infected for 18 hours with *N. caninum* tachyzoites (NcLiv; MOI 0.5) or stimulated for 3 hours with 500 ng/mL LPS (TLR4/Caspase-11 agonist) and 2.5 μM ATP (NLRP3 inducer) for 1 hour. Caspase-1/11 activity was measured by fluorescent signal in a plate reader using fluorescent FAM-YVAD-FMK (FLICA) probe (A); IL-1β production was measured by ELISA (B); pore formation was quantified by propidium iodide (PI) incorporation (C); and ROS production was measured by the fluorescent DHCFDA probe (D). Values are representative of two independent experiments and each condition was conducted at least in triplicates. Values indicating mean ± SEM of cytokine levels in relation the standard curve and fluorescence levels (relative fluorescence units – RFU) (*P < 0.05; ANOVA with the Bonferroni multiple comparison post-hoc test between naïve and *N. caninum* infected BMDMs).

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3